



CALIFORNIA DEPARTMENT OF HEALTH SERVICES (CDHS) GUIDELINES FOR COLLECTING AND SHIPPING SPECIMENS FOR INFLUENZA A (H5N1) DIAGNOSTICS

(Adapted from the 2005 US Department of Health & Human Services Pandemic Influenza Plan)

******CALIFORNIA LOCAL HEALTH DEPARTMENTS AND CDHS MUST BE NOTIFIED ABOUT ANY CASE OF SUSPECT AVIAN INFLUENZA A (H5N1)******

Many local public health laboratories in California can perform polymerase chain reaction (PCR) and subtype testing. Local health departments should be contacted to determine where specimens should be submitted. Local health departments should report any suspect or laboratory-confirmed case to CDHS immediately.

To submit specimens to CDHS VRDL:

1. All specimens should be labeled with the following information: PATIENT'S NAME, DATE TAKEN AND TYPE OF SPECIMEN. Because culture is not recommended in these cases, please note on the form that this is a suspect case of avian influenza a (H5N1).
2. Complete a CDHS Viral and Rickettsial Disease Laboratory Specimen Submittal Form for Suspect Avian Influenza A (H5N1) for each vial with the following information: patient's name, age, date of onset of illness, type of specimen, date collected and clinical symptoms.
3. For questions about specimen submittal, contact David Cottam at 510 307-8585.

SPECIMEN COLLECTION

To improve diagnostic sensitivity, testing should be performed on multiple samples types. Some studies have demonstrated the need for multiple samples collected over several days for optimal H5 detection sensitivity. Given that most human cases have presented with lower respiratory tract infections, the collection of only a upper respiratory specimen, particularly single nasopharyngeal or nasal swabs, is NOT recommended.

MINIMUM SPECIMEN REQUIREMENTS FOR INFLUENZA A (H5N1) TESTING INCLUDE THE FOLLOWING:

- 1) A nasopharyngeal swab OR nasopharyngeal wash OR nasopharyngeal aspirate collected in 3 cc viral transport media (VTM); AND
- 2) a throat swab collected in 3 cc viral transport media (VTM); AND
- 3) any specimen(s) from the lower respiratory tract (e.g., sputum, bronchoalveolar lavage, tracheal aspirate or pleural fluid tap)*.

- * In outpatient settings, it may be difficult to obtain samples from the lower respiratory tract in children. In these instances, two specimens from the upper respiratory tract (e.g. a nasopharyngeal wash and a throat swab) are acceptable.

I. RESPIRATORY SPECIMENS

Respiratory specimens are optimally collected within the first 3 days of illness onset.

A. Collecting specimens from the upper respiratory tract

1. Nasopharyngeal wash/aspirate

- Have the patient sit with head tilted slightly backward.
- Instill 1 ml–1.5 ml of nonbacteriostatic saline (pH 7.0) into one nostril. Flush a plastic catheter or tubing with 2 ml–3 ml of saline. Insert the tubing into the nostril parallel to the palate. Aspirate nasopharyngeal secretions. Repeat this procedure for the other nostril.
- Collect the specimens in sterile vials.
- For shipping, use cold packs to keep the sample at 4°C.

2. Nasopharyngeal or oropharyngeal swabs

- Use only sterile dacron or rayon swabs with plastic shafts. Do **not** use calcium alginate or cotton swabs or swabs with wooden sticks, as they may contain substances that inactivate some viruses and inhibit PCR testing.
- To obtain a **nasopharyngeal swab**, insert a swab into the nostril parallel to the palate. Leave the swab in place for a few seconds to absorb secretions. Swab both nostrils.
- To obtain an **oropharyngeal swab**, swab the posterior pharynx and tonsillar areas, avoiding the tongue.
- Place each swab immediately into two separate sterile vials containing 2 ml of viral transport media (VTM, either commercially available, herpes buffere tryptose gelatin meium or Hanks' balanced salt solution with gelatin). Break the applicator sticks off near the tip to permit tightening of the cap.
- For shipping, use cold packs to keep the sample at 4°C.

B. Collecting specimens from the lower respiratory tract

1. Bronchoalveolar lavage, tracheal aspirate, or pleural fluid tap

- During bronchoalveolar lavage or tracheal aspirate, use a double-tube system to maximum shielding from oropharyngeal secretions.
- Place the unspun fluid in sterile vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®.
- For shipping, use cold packs to keep the sample at 4°C.

2. Sputum

- Educate the patient about the difference between sputum and oral secretions.
- Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile screw-cap sputum collection cup or sterile dry container.
- For shipping, use cold packs to keep the sample at 4°C.

II. BLOOD COMPONENTS (optional)

Collection of sera for serologic testing for influenza as well as other respiratory viruses can be considered, but should not replace collection of respiratory specimens, which are highly recommended for influenza A (H5N1) testing. For serologic testing, paired blood samples are ideal. Collect an acute phase blood specimen (5-10 ml whole clotted blood) on each patient, complete a CDHS Viral and Rickettsial Disease Specimen Submittal Form for Suspect Avian Influenza A (H5N1), and schedule patient to return in 14-21 days for a convalescent blood specimen.

To collect serum for antibody testing:

- Collect 5 ml–10 ml of whole blood in a serum separator tube. Allow the blood to clot, centrifuge briefly, and collect all resulting sera in vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®.
- The minimum amount of serum preferred for each test is 200 microliters, which can easily be obtained from 5 ml of whole blood. A minimum of 1 cc of whole blood is needed for testing of pediatric patients. If possible, collect 1 cc in an EDTA tube and in a clotting tube. If only 1cc can be obtained, use a clotting tube.
- If unfrozen, ship with cold packs to keep the sample at 4°C. If frozen, ship on dry ice.

III. AUTOPSY SPECIMENS

Immunohistochemical (IHC) staining for influenza A (H5) viruses can be performed on autopsy specimens at the Centers for Disease Control and Prevention. Viral antigens may be focal and sparsely distributed in patients with influenza, and are most frequently detected in respiratory epithelium of large airways. Larger airways (particularly primary and segmental bronchi) have the highest yield for detection of influenza viruses by IHC staining. Collection of the appropriate tissues ensures the best chance of detecting the virus by (IHC) stains.

- If influenza is suspected, a minimum total of 8 blocks or fixed-tissue specimens representing samples from each of the following sites should be obtained and submitted for evaluation:
 - Central (hilar) lung with segmental bronchi
 - Right and left primary bronchi
 - Trachea (proximal and distal)
 - Representative pulmonary parenchyma from right and left lung

In addition, representative tissues from major organs should be submitted for evaluation. In particular, for patients with suspected myocarditis or encephalitis, specimens should include myocardium (right and left ventricle) and CNS (cerebral cortex, basal ganglia, pons, medulla, and cerebellum). Specimens should be included from any other organ showing significant gross or microscopic pathology.

- Specimens may be submitted as:
 - Fresh-frozen unprocessed tissue in 10% neutral buffered saline, or
 - Tissue blocks containing formalin-fixed, paraffin-embedded specimens, or
 - Unstained sections cut at 3 microns placed on charged glass slides (10 slides per specimen)

- Specimens should be sent at room temperature (**NOT FROZEN**).
- Fresh-frozen unfixed tissue specimens may be submitted for RT-PCR.
- Include a copy of the autopsy report (preliminary, or final if available), and a cover letter outlining a brief clinical history and the submitter's full name, title, complete mailing address, phone, and fax numbers.